Specialty Conference

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An edited transcription of an Interdepartmental Clinical Case Conference arranged by the Department of Medicine of the UCLA School of Medicine (University of California, Los Angeles)

Refer to: Paulus HE, Machleder HI, Clements PJ, et al: Prolonged thoracic-duct drainage in rheumatoid arthritis and systemic lupus erythematosus—Interdepartmental Clinical Case Conference, University of California, Los Angeles (Specialty Conference). West J Med 130:309-324, Apr 1979

Prolonged Thoracic-Duct Drainage in Rheumatoid Arthritis and Systemic Lupus Erythematosus

The thoracic duct was surgically cannulated in nine patients with rheumatoid arthritis and in two patients with systemic lupus erythematosus. Lymph was drained continuously for up to 105 days, lymphocytes were removed by centrifugation and the cell-free lymph was reinfused intravenously. Diversion of thoracic-duct lymphocytes produced a prompt decrease in synovitis and nodule size in rheumatoid arthritis, a diminution of cutaneous vasculitis and proteinuria in systemic lupus erythematosus and a marked drop in IgG serum concentrations. Prolonged thoracic-duct drainage with depletion of recirculating lymphocytes resulted in a pronounced decrease in delayed hypersensitivity, prolongation of skin-graft survival, selective suppression of humoral antibody responses and additional clinical improvement. Thoracic-duct T cells were depleted slightly more than B cells. There was no effect on responses to induced inflammation. These findings are consistent with the hypothesis that some of the drained cells are essential participants in the inflammatory manifestations of rheumatoid arthritis and systemic lupus erythematosus in these patients.

Supported in part by USPHS Grants GM 15759, RR 865 and the Thomas and Dorothy Leavey Trust.

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HAROLD E. PAULUS, MD:* Many diseases of unknown cause are considered to be autoimmune; most of the rheumatic diseases are included in this category. So-called immunosuppressive drugs have been used with some success in the treatment of various autoimmune diseases,[1-4] but the risk involved in using these cell poisons is high. Moreover, we cannot confidently attribute beneficial effects to a single action of the immunosuppressive drugs because these agents may act on a multiplicity of sites.

According to some reports, thoracic-duct drainage (TDD) has permitted successful cadaver renal transplantation when used as the sole immunosuppressive therapy.[5-7] Its effects on immune responses have also been studied in experimental animals.[8-11]

In this conference we shall discuss the use of thoracic-duct drainage as a tool to study the effects of this method of immunosuppression in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). We shall characterize the general effects of thoracic-duct drainage on various aspects of cell-mediated and humoral immune responses, as well as its effects on induced inflammation. In addition, the effect of thoracic-duct drainage

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ABBREVIATIONS USED IN TEXT
ANA = antinuclear antibody
β_1C = Beta-1-C component of complement
Con A = concanavallin A
DNCB = dinitrochlorobenzene
E-RFC = lymphocytes forming rosettes with sheep red blood cells
HL-A = human leukocyte-locus A
KLH = keyhole-limpet hemocyanin
PHA = phytohemagglutinin
RA = rheumatoid arthritis
SIg = surface membrane immunoglobulin
SLE = systemic lupus erythematosus
SRBC = sheep red blood cells
TDD = thoracic-duct drainage

on the inflammatory manifestations and on some autoimmune markers of systemic lupus erythematosus and rheumatoid arthritis will be presented.

In order to understand thoracic-duct drainage, it is necessary to review the anatomy of lymphocyte circulation (Figure 1). Lymphocytes from the bone marrow and the thymus enter and circulate in the blood. Some lymphocytes migrate through the walls of postcapillary venules directly into lymph nodes. It is presumed that they can also leave the blood and travel through the tissues until they arrive at a lymph node by way of its afferent lymphatics. Antigenic stimulation of lymphocytes may occur in the tissues or in the lymph nodes, and lymphocyte proliferation and recruitment may occur in the nodes. Ultimately many lymphocytes leave the nodes by way of efferent lymphatics, most of which become tributaries of the thoracic duct. The contents of the thoracic duct are then returned to the peripheral blood, and the cycle starts over again. The spleen acts like an intravascular lymph node. Cells leaving the spleen enter the peripheral blood directly, but the rest of the lymphocyte circuit is unidirectional. After a lymphocyte leaves the circulation, it can return only by way of the thoracic duct or by a few of the other major lymphatic vessels. Because the thoracic-duct lymph contains no platelets and very few neutrophils and eosinophils, and has no easily recognizable monocytes, it provides a convenient mechanical separation of lymphocytes from other blood cells.

When we cannulate the thoracic duct, we divert recirculating small lymphocytes and lymphocytes that have been recently antigen-stimulated or newly proliferated, thereby preventing their entry into the bloodstream. This diversion may have immediate effects to the extent that diverted cells are unable to fulfill their function.

A second major effect occurs with prolonged

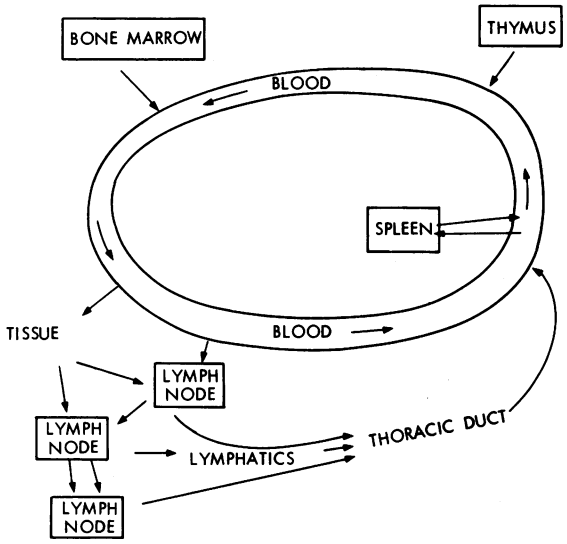

Figure 1.—Anatomy of lymphocyte circulation.

thoracic-duct drainage. If the drained lymphocytes are not returned to the patient, the body's pool of recirculating lymphocytes is gradually depleted, particularly of long-lived (and slowly replaced) recirculating lymphocytes. Therefore, in discussing the effects of thoracic-duct drainage, we shall attempt to distinguish the early effects of lymphocyte *diversion* from the later effects of lymphocyte *depletion.*

Surgical Technique

HERBERT I. MACHLEDER, MD:* The technique of thoracic-duct drainage has been described previously.[12-14] However, premature closure of the fistulae because of technical difficulties has often limited its usefulness both as an investigative tool in the study of autoimmune phenomena and as a therapeutic modality to effect immunosuppression by means of lymphocyte depletion. By carefully modifying several steps in this procedure, it has been possible to prolong the longevity of thoracic-duct fistulae in such a fashion that they can be used in clinical protocols.

The thoracic duct (Figure 2) arises at the cisterna chyli, the confluence of all the infradiaphragmatic lymphatics. It ascends the posterior mediastinum and enters the cervical region in the retroesophageal position. At this point it curves, much as the handle of a cane, goes beneath the left internal jugular vein and unites with a plexus of brachiocephalic lymphatic trunks to form a dilated ampulla at the junction of the left subclavian vein and the left jugular vein. The duct is most accessible for cannulation in this area;

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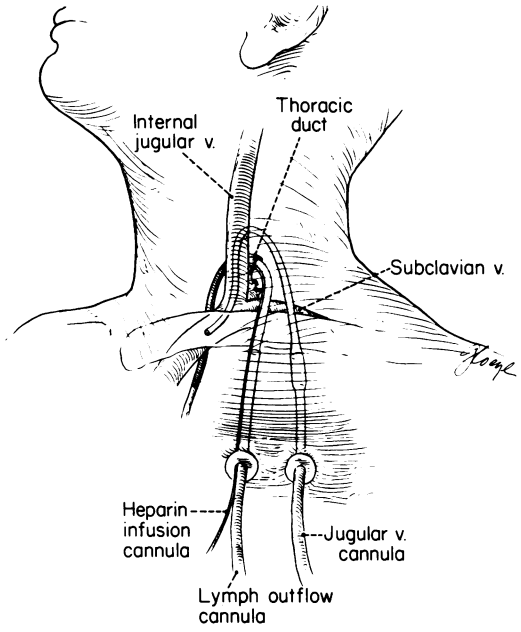

Figure 2.—Surgical anatomy of terminal thoracic duct.

it lies in the scalene anticus fat pad just beneath the clavicular belly of the sternocleidomastoid muscle.

The entire operation is done above the clavicle in the left side of the neck. An incision is made parallel to the clavicle and continued deep to the sternocleidomastoid muscle. After the jugular vein is carefully mobilized, the ampulla is usually easily visible as it enters the jugular subclavian junction. The ampulla is dissected and ligated at its most distal portion; lymph then distends the thoracic duct and the collateral channels that join at this confluence. The collateral channels must be carefully ligated to prevent diversion of lymph from the thoracic-duct fistula, which would otherwise occur after a period of time. We have found that one of the major causes of failure in an initially successful thoracic-duct fistula has been the enlargement of collateral channels that divert the lymph into new lymphovenous communications. If thoracic-duct drainage output begins to fall, retrograde thoracic-duct lymphangiography may show these collateral channels, which can then be ligated, usually under a local anesthetic. The lymph flow then promptly returns to its usual output volume. This drainage problem can be prevented by carefully identifying the collateral channels at the time of the initial operation and checking that they are securely ligated.

After the thoracic duct has been isolated, an incision is made in the duct wall, and probes are

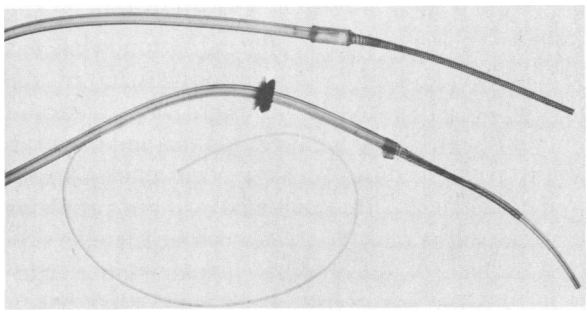

Figure 3.—Components needed for thoracic-duct cannulation. **Bottom:** Soft silastic, double-lumen, wire-wound thoracic-duct cannula; heparin solution is infused through the smaller lumen, and heparinized lymph flows out through the larger lumen. The exit button has been placed on the cannula approximately where the cannula penetrates the skin. **Top:** Soft silastic venous cannula; a valve at the tip of the tube prevents retrograde flow of blood.

passed in a retrograde fashion to disrupt the sets of semilunar valves that line the proximal thoracic duct and prevent regurgitation of blood into the thoracic duct. Unless these valves are carefully disrupted, they will prevent the passage of the soft silastic cannula deep enough into the thoracic duct to establish a secure fistula. The cannulae we have designed (Figure 3) have a double lumen and are made of soft medical-grade silastic. One lumen allows continuous heparin infusion to the tip of the catheter and into the thoracic duct; the second lumen allows free exit of heparinized lymph. A wire coil is embedded in the wall of the cannula and extends from the tip of the tube to include the entire portion that will remain in the subcutaneous tissue. This coil prevents kinking of the tube and obstruction to lymph flow by flexion, extension or head movements that may cause the tube to bend or change its position. In order to maintain a long-term thoracic-duct fistula, it is essential to use a tube that will remain patent but whose composition elicits minimal tissue reaction. Although the very soft medical-grade silastic fulfills the purpose of tissue compatibility, it is easily obstructed. The coiled wire structure within its wall maintains the patency of the tube. The double-lumen tube* is ligated in place in the thoracic duct and is further secured with several other sutures to the scalene fat pad. The tube is then passed through a subcutaneous tunnel over the clavicle and through an exit site in the skin several centimeters inferior to the clavicle. At this point, it exits through a button* consisting of

*Manufactured by Heyer-Shulte Company, Santa Barbara, California.

a porous Teflon pad with a silastic collar. The tube is cemented to this silastic button, which is gradually infiltrated with fibrous tissue in the subcutaneous space, and thus provides a secure point of fixation and a barrier to fluid and bacteria. With this procedure we have been able to maintain cannulae for three months without problems of local sepsis or skin breakdown at the exit site.

A branch of the internal jugular vein is cannulated with a second silastic tube that has a single lumen. This tube is passed through a similar subcutaneous tunnel and is brought out in an infraclavicular position adjacent to the thoracic-duct cannula. This jugular line is used only for the reinfusion of cell-free lymph. The lymph is collected in an iced glass flask set in a movable cart, thereby enabling the patient to be completely ambulatory. Each morning the flask is changed, and the lymph is processed under sterile conditions, using a laminar flow hood and a continuous-flow centrifuge to separate the lymph from the lymphocytes. Cultures of the cell-free lymph are done daily and the lymph is stored under refrigeration until the cultures have been determined to be negative. The lymph is then reinfused through the jugular line from an intravenous pole attached to the movable cart. During drainage, the patient is encouraged to walk; he is able to move about the hospital in a relatively unrestricted fashion for laboratory tests, physical therapy and other requirements.

DR. PAULUS: We have thus established a system for continuous collection of lymph, removal of cells and reinfusion of sterile cell-free lymph that can be continued for many weeks. We have also seen a characteristic time-course of changes occurring during thoracic duct drainage.

Effects of Thoracic-Duct Drainage on Lymphocyte Concentrations and Characteristics

Daily lymphocyte output ranges from 10×10^9 to 50×10^9 early in drainage but decreases as drainage continues, usually stabilizing after three to six weeks at 1×10^9 to 5×10^9. Total lymphocyte output varies with persons and with duration of drainage; the maximum to date has been about 10^{12} cells from a patient whose drainage continued for 102 days. Lymphocyte concentrations in peripheral blood decrease during the first four weeks and then remain at rather low but stable levels. After thoracic-duct drainage, it takes about 15 weeks for the concentration to return to baseline levels. Early in drainage, the cells are almost all small dense lymphocytes. After one to four weeks, larger, lighter cells begin to appear, which often have nucleoli and are sometimes seen in mitotic division.

As the larger cells are less dense, it is convenient to follow their concentrations by discontinuous density gradient centrifugation. When this is done, there has been a progressive decrease in high-density cells associated with an increase in low-density cells during the first eight weeks of thoracic-duct drainage. Surprisingly, there is no evidence of hyperplasia of lymphoid precursors in the bone marrow, although peripheral blood lymphocytes also become larger and less dense.

PHILIP J. CLEMENTS, MD:* In collaboration with Dr. David Yu, we evaluated surface membrane receptors on thoracic-duct lymphocytes and on peripheral blood lymphocytes before and during thoracic-duct drainage. Table 1 shows the results

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TABLE 1.—*Percentages of Lymphocyte Subpopulations as Defined by Various Cell Surface Receptors on Lymphocytes From the Thoracic Duct and the Peripheral Blood of Patients With Severe Rheumatoid Arthritis or Scleroderma**

	Lymphocytes		
	TD†	*PB*	
Cell Surface Receptors	*7 RA and 2 PSS (Percent)*	*11 RA (Percent)*	*11 PSS (Percent)*
Binding sheep red blood cells (T lymphocytes)	60±4	76±3	74±4
Surface Ig (B lymphocytes)			
All Ig classes	24±3	12±2	14±2
IgM only	21±6	10±2	12±2
Binding aggregated IgG (B and L lymphocytes)	27±4	20±2	22±2
Complement receptor (B and L lymphocytes)	29±6	13±1	9±2

PB = peripheral blood; PSS = scleroderma; RA = rheumatoid arthritis; TD = thoracic duct

*All studies were not done in every subject.
†Examined within first 14 days of drainage.

for several membrane receptors as identified on thoracic-duct lymphocytes of nine patients within their first 14 days of drainage. For comparison, the same receptors were evaluated on peripheral-blood lymphocytes from a group of patients with severe rheumatoid arthritis or scleroderma. The values for peripheral blood are not significantly different from those of normal persons previously reported from this laboratory.[15,16]

T lymphocytes were identified by their ability to form spontaneous rosettes with sheep red blood cells (E-RFC), and B lymphocytes were enumerated by immunofluorescent staining of surface membrane immunoglobulin (SIg).[17] Before the staining of surface membrane immunoglobulin with fluorescein-conjugated goat antisera to human immunoglobulin, the cells were incubated at 37°C for 30 to 60 minutes in media free of human serum to induce shedding of any adsorbed or cytophilic immunoglobulin.[18]

Using monospecific fluorescent goat antisera to human immunoglobulins, the IgM class of immunoglobulins accounted for most of the surface membrane immunoglobulin on both thoracic-duct lymphocytes and peripheral blood lymphocytes in this study, as it has in other recent investigations.[19]

By means of an indirect immunofluorescent method, binding of heat-aggregated human IgG was used to identify Fc-receptors (the Fc portion of the antibody molecule) on lymphocytes. By using a combination of immunofluorescent and rosette techniques concurrently, we have been able to identify receptors for aggregated IgG on most B lymphocytes, as well as on a separate population called K or L lymphocytes.[20] Some investigators feel that these lymphocytes may be largely responsible for antibody-dependent cellular cytotoxicity.[18,19,21-23] As K or L lymphocytes do not have surface membrane immunoglobulin and do not rosette with sheep red blood cells, they may represent a subset or grouping distinct from the classical groupings of B and T lymphocytes.[18,19,22,23] Lymphocytes with receptors for the third component of complement were assessed by a rosetting technique using complement-coated zymosan granules.[16] We have found complement receptors primarily on circulating B and L lymphocytes. Only a few circulating T lymphocytes (as assessed by rosetting with sheep red blood cells) also form rosettes with complement-coated zymosan granules.

In several animal systems, T lymphocytes that have become activated or "turned-on" have been shown to develop Fc-receptors.[24,25] It has been suggested that in the autoimmune diseases there may be a greater than normal number of T lymphocytes that have become activated as part of the disease. We therefore searched for such activated T lymphocytes in thoracic-duct lymph and

TABLE 2.—*Receptors for Sheep Red Blood Cells Occurring Simultaneously on Lymphocytes With Receptors for Aggregated IgG or With Surface Immunoglobulin*

Source	Number of Patients	Agg and E-RFC			SIg and E-RFC		
		Total Agg	Total E-RFC	Both Simultaneously	Total SIg	Total E-RFC	Both Simultaneously
Thoracic-duct lymph	3	60.8±15.7	38.7±15.8	4.1±2.1	52.8±15.1	38.4±17.0	2.9±2.2
Blood	9*	19.8± 2.2	73.1± 2.8	3.6±0.6			

Agg = aggregated IgG
E-RFC = lymphocytes forming rosettes with sheep red blood cells
SIg = surface immunoglobulin

*With classical rheumatoid arthritis.

TABLE 3.—*Changes in Absolute Lymphocyte Concentrations and in Percentages of Cells Binding Aggregated IgG or Sheep Red Blood Cells in Lymph and Blood Before and During Thoracic-Duct Drainage*

Drainage Duration	Lymph*			Blood*		
	Lymphocyte Concentration (Per cu mm)	Agg (Percent)	E-RFC (Percent)	Lymphocyte Concentration (Per cu mm)	Agg (Percent)	E-RFC (Percent)
Before drainage	..	..	..	1,690±291	21±2	70±6
Week 1	8,090±1,170	30±6	58±3	1,223±162	..	..
Week 2	6,530±1,350	28±7	62±8	811±142	20±4	..
Week 3	6,500±1,350	30±6	56±3	718± 99	..	..
Week 4	4,500±1,080	38±6	51±4	611±101	24±3	70±7

Agg = aggregated IgG
E-RFC = lymphocytes forming rosettes with sheep red blood cells

*Total number of patients: lymph, 11; blood, 7.

TABLE 4.—*Changes in Absolute Lymphocyte Concentrations and in Percentages of Cells Binding Aggregated IgG or Sheep Red Blood Cells in Lymph During Prolonged Thoracic-Duct Drainage**

Drainage Duration	Lymphocyte Concentration (Per cu mm)	Agg (Percent)	E-RFC (Percent)
Week 1	11,300±1,100	34±11	59± 5
Week 2	7,970± 920	40±13	..
Week 3	7,600±3,290	46±13	48± 8
Week 4	3,130± 670	47±13	44± 6
Week 7	1,330± 280	42±13	48±12
Weeks 12-14 ..	1,670± 780	52± 5	41± 6

Agg = aggregated IgG
E-RFC = lymphocytes forming rosettes with sheep red blood cells

*In three patients drainage continued for up to 14 weeks.

in peripheral blood by assaying lymphocytes simultaneously for binding of aggregated IgG and for binding of sheep red blood cells. The results (Table 2) suggested, however, that only a small percentage of cells from lymph (4.1 percent) or blood (3.6 percent) bound both sheep red blood cells and aggregated IgG simultaneously. Other experiments also showed that cells with surface membrane immunoglobulin were a population of cells different from those that bound sheep red blood cells.

Serial assays from blood and lymph of absolute lymphocyte counts were done and percentages of lymphocytes binding aggregated IgG or sheep red blood cells were calculated during the first four weeks of drainage in 11 patients (Table 3). During drainage, a progressive absolute lymphopenia occurred in both blood and lymph. Moreover, in lymph there was a relative increase in the proportion of B and L lymphocytes, with a reciprocal decrease in T lymphocytes. In blood, however, there was no change in the relative proportions of these two subpopulations.

Three patients were studied for up to 14 weeks of thoracic-duct drainage (Table 4). The previously established trend toward increasing percentages of B and L lymphocytes and decreasing percentages of T lymphocytes continued throughout the thoracic-duct drainage, despite the fact that the absolute lymphocyte count had stabilized by the seventh week. Because of the pronounced lymphopenia, peripheral blood assays were not routinely done after the first month of drainage.

The responses of thoracic-duct lymphocytes to several mitogens were also evaluated serially in three patients by Dr. James B. Peter and Dr. David Yu by methods previously published.[26] The mitogens evaluated included phytohemagglutinin (PHA), pokeweed mitogen, and concanavallin A (Con A). Although the lymphocyte response to mitogens was low in the first few days of thoracic-duct drainage, there was a significant increase in response to mitogens thereafter. This increase was several times greater than the predrainage response of peripheral blood lymphocytes of the patients—or, for that matter, of normal persons. We had anticipated that the response of thoracic-duct lymphocytes to mitogens would have slowly decreased during drainage. Such a decrease in response was seen in the first patient, but in the other two patients in whom drainage continued for up to eight weeks, no such consistent downward trend occurred. We conclude, therefore, that in general there was no consistent change in the level of responses to mitogens during drainage.

Effects of Thoracic-Duct Drainage on Host Immune Responses

Skin Homograft Survival

DR. MACHLEDER: Although there has been extensive documentation of prolonged homograft survival with various immunosuppressive modalities, this study permitted us to determine whether prolongation of homograft survival could be achieved when thoracic-duct lymphocyte depletion was the sole immunosuppressive measure.[27] In seven patients who underwent lymphocyte depletion by prolonged thoracic-duct drainage, full-thickness skin grafts from nonconsanguinous donors were placed in a defect created in the anterior thigh of the patient. These homografts were regularly inspected; and in two cases, where the graft was from a donor of the opposite sex, the chromosomal characteristics of the healed skin graft were verified by biopsy. The survival of the skin homografts varied from 8 to 550 days. The duration of survival did not appear to be correlated with the duration of thoracic-duct drainage or with the number of lymphocytes depleted. A relationship was established, however, between the duration of survival of the skin homograft and the residual lymphocyte output. The residual lymphocyte output represents the extent of lymphocyte depletion and is calculated by dividing the average daily lymphocyte output during the final week of thoracic-duct drainage by the average daily lymphocyte output during the first week of drainage; the result is expressed as a percentage. The patient whose graft survived eight days had a residual lymphocyte output of 80 percent, suggesting that successful depletion of lymphocytes had not been achieved despite 80 days of drainage; a large

number of lymphocytes were still being recovered in the thoracic-duct lymph at the end of thoracic-duct drainage. On the other hand, four patients whose grafts survived between 34 and 550 days had residual lymphocyte outputs of 22 percent or less. The longest surviving graft was in a patient whose residual lymphocyte output was 7 percent.

In one patient in whom the most profound immunosuppression was achieved, it is likely that immunologic tolerance developed to a D matched skin homograft. Two successive skin homografts from the same donor showed prolonged survival with no evidence of rejection. Cytogenic studies of biopsy material from both grafts revealed X-Y male karyotypes of the donor cells. The skin homografts remained intact, although other immune responses (including delayed hypersensitivity skin tests, humoral immune responses and peripheral lymphocyte counts) had returned to normal, and disease activity had reappeared at least six months before the second graft was done. To further assess the patient's immunologic tolerance, a third skin homograft, from a second male donor, was placed on the opposite thigh. The HL-A (human leukocyte-locus A) tissue typing of this graft showed two subloci of incompatibility both with the recipient and the previous donor. Although the skin graft that had been placed during thoracic-duct drainage survived 550 days and a second identical graft placed after return of immunologic competence survived 325 days, the third skin graft was rejected in 14 days. It seems evident that immunologic tolerance developed only to the skin graft that was placed during thoracic-duct drainage. It seems likely that a point is reached during prolonged thoracic-duct drainage when the patient's immunologic competence is considerably attenuated. It remains to be established whether there exists a certain level of immunosuppression at which point predictable and reproducible tolerance to transplanted tissue can be expected.

Delayed Hypersensitivity

DR. PAULUS: We can therefore conclude that skin transplant survival seems to be a function of lymphocyte *depletion,* rather than of thoracic-duct lymphocyte *diversion.* Similarly, delayed hypersensitivity skin test responses to both primary and secondary antigens were diminished after four to six weeks of thoracic-duct drainage. Dinitrochlorobenzene (DNCB) immunization was successful at the end of thoracic-duct drainage in only two of six patients. Intradermal injection of recall antigens produced 15.3 ± 2.3 mm of induration at baseline, but only 2.3 ± 1.5 mm after 32 days of thoracic-duct drainage.

Humoral Immunity

The effect of thoracic-duct drainage on humoral immunity was evaluated in several ways. The primary antigen keyhole-limpet hemocyanin (KLH) was given *at the end* of thoracic-duct drainage. The antibody response[28,29] of the drained patients was comparable with that of 36 control patients with rheumatoid arthritis who were being treated with nonsteroidal anti-inflammatory drugs (Figure 4).

Tetanus toxoid was given as a recall antigen

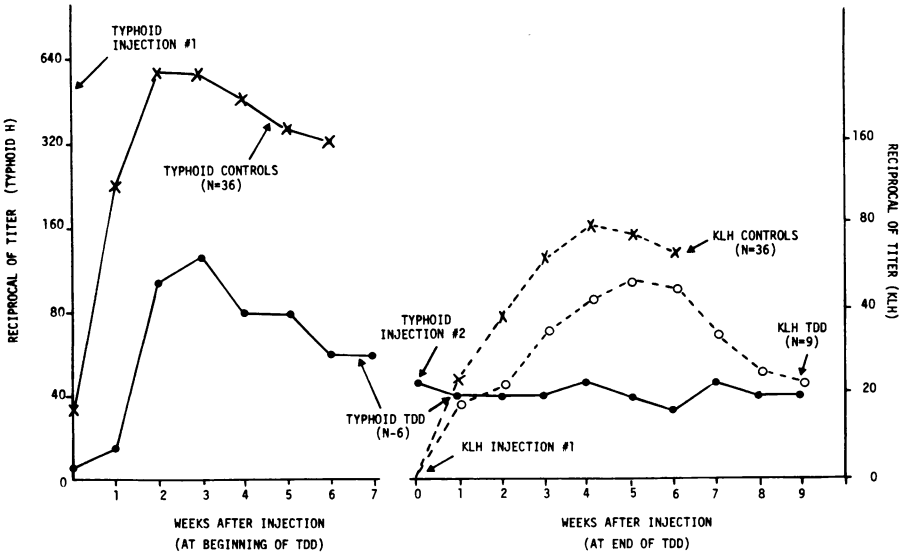

Figure 4.—Antibody responses to the primary antigen keyhole-limpet hemocyanin (KLH) and the recall antigen typhoid H. TDD = thoracic-duct drainage.

both at the beginning and at the end of thoracic-duct drainage; the initial antibody response[30] was similar to that of the controls. The second injection at the end of drainage also increased antibody titer, although the response was not as great as might have been expected.

With typhoid O antigen, there was only a barely perceptible increase in titer after the second injection, given at the end of thoracic-duct drainage, whereas with typhoid H antigen there was no increase (Figure 4).

Immunoglobulins

Serum immunoglobulin concentrations were also studied. During the first week of thoracic-duct drainage, IgG concentration decreased strikingly from 1,450 to 980 mg per dl, with little subsequent change, as compared with concentrations found in control patients with rheumatoid arthritis. Surprisingly, no change in IgM concentration was seen during six weeks of thoracic-duct drainage. IgA concentrations were lower at baseline in the patients undergoing thoracic-duct drainage than in controls with rheumatoid arthritis, and they gradually decreased by about 40 percent during six weeks of drainage. In order to prevent protein depletion in the patients, their cell-free lymph was returned to them daily. Serum albumin remained constant, and the decrease in total protein reflected the changes in immunoglobulin concentrations. Immunoglobulin synthesis rate was examined in one patient by Levy and co-workers.[31] The synthesis of both IgG and IgM was abnormally high before thoracic-duct drainage but was normal when evaluated again at the end of drainage, suggesting that the observed changes in serum concentration are, at least partly, due to decreased synthesis.

Induced Inflammation

Could some of the effects of thoracic-duct drainage be the result of general effects on inflammatory responses rather than of specific effects on immunity? We examined several assays of induced inflammation (Trafuril erythema,[32] microcrystalline sodium urate injections,[33] Rebuck skin windows[34]) before and at the end of thoracic-duct drainage and found no change in them. There was also no effect on marrow granulocyte reserves as measured by etiocholanolone responses[35] or on lymphocyte concentrations in bone-marrow aspirates.

Effects of Thoracic-Duct Drainage on Disease Manifestations

Systemic Lupus Erythematosus

KENNETH NYMAN, MD:* Many of the findings in systemic lupus erythematosus, including cutaneous vasculitis and glomerulonephritis, are thought to be mediated by the deposition of immune complexes. Deposition of immune complexes and complement in blood vessel walls and at the dermal-epidermal junction is found both in lesion tissue and in uninvolved skin of patients with systemic lupus erythematosus.[36,37] The disappearance of immunoglobulin deposits at the dermal-

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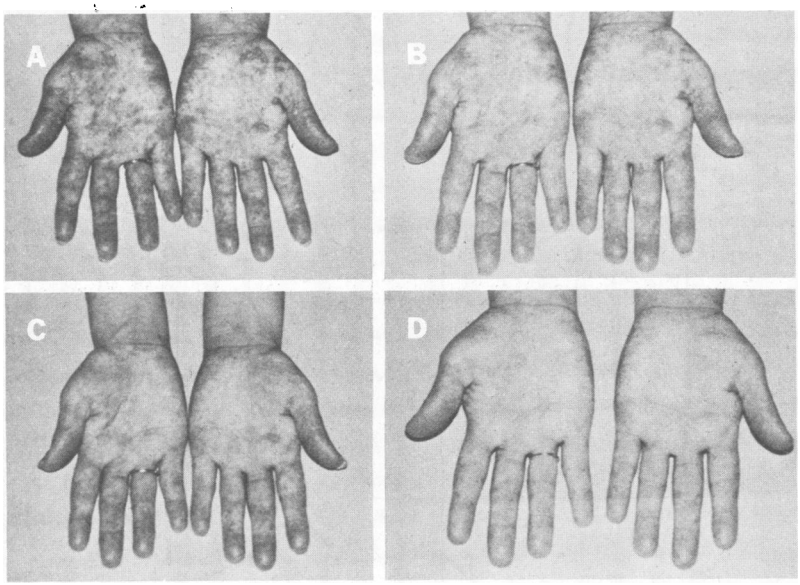

Figure 5.—Serial photographs of patient with systemic lupus erythematosus cutaneous vasculitis. **A:** one week after beginning thoracic-duct drainage; **B:** at end of second week of drainage; **C:** in seventh week; **D:** 11 weeks after end of thoracic-duct drainage.

epidermal junction has been noted in the uninvolved skin of patients with systemic lupus erythematosus treated with cyclophosphamide.[38] It has been suggested that this may serve as a useful indicator of the effectiveness of therapy. As we have stated, prolonged lymphocyte depletion has been shown to produce a significant immunosuppressive effect.

In view of these observations, we decided to study the effects of thoracic-duct drainage, as a noncytotoxic procedure, in a 25-year-old woman with a four-year history of systemic lupus erythematosus manifested primarily by cutaneous vasculitis.[39] She was only partially and intermittently responsive to very high doses of oral corticosteroids and azathioprine. Biopsy studies of skin gave findings consistent with systemic lupus erythematosus. Other manifestations of the patient's disease included lupus nephritis (predominantly membranous), recurrent serositis, pancreatitis, fever, arthralgias and seizures. From September 1974, she had manifested incapacitating vasculitis involving buccal and nasal mucosa, and the skin of face, forearms and hands (including the nails) (Figure 5). Because of failure to control the patient's disease activity with administration of 100 mg of prednisone and 200 mg of azathioprine per day, and because of the recognized dangers and complications associated with long-term oral administration of corticosteroid and azathioprine, we decided to initiate thoracic-duct drainage.

On December 2, 1975, the thoracic duct was surgically cannulated. The volume of lymph in any one 24-hour period ranged from 1,100 to 3,500 ml; the number of lymphocytes drained per day ranged from 200 million cells to 33 billion cells. At the end of ten weeks, 277×10^9 lymphocytes had been drained from the patient.

After one week of thoracic-duct drainage, the patient's condition improved considerably (Figure 5). During drainage, there was no clinical, laboratory or histological evidence of change in the patient's lupus nephritis, and the dosage of prednisone was tapered to 60 mg every other day. She has subsequently been followed as an outpatient, and she was maintained on this amount of prednisone with no evidence of disease activity until July 8, 1976, when a vasculitic lesion was noted on her hard palate. With prolonged drainage the immunoglobulin deposition in the skin decreased.

After ten days of thoracic-duct drainage, examination of skin biopsy specimens showed 3+ continuous fluorescence for β_1C and, after six weeks, 2+ focal deposition. Subsequently, there

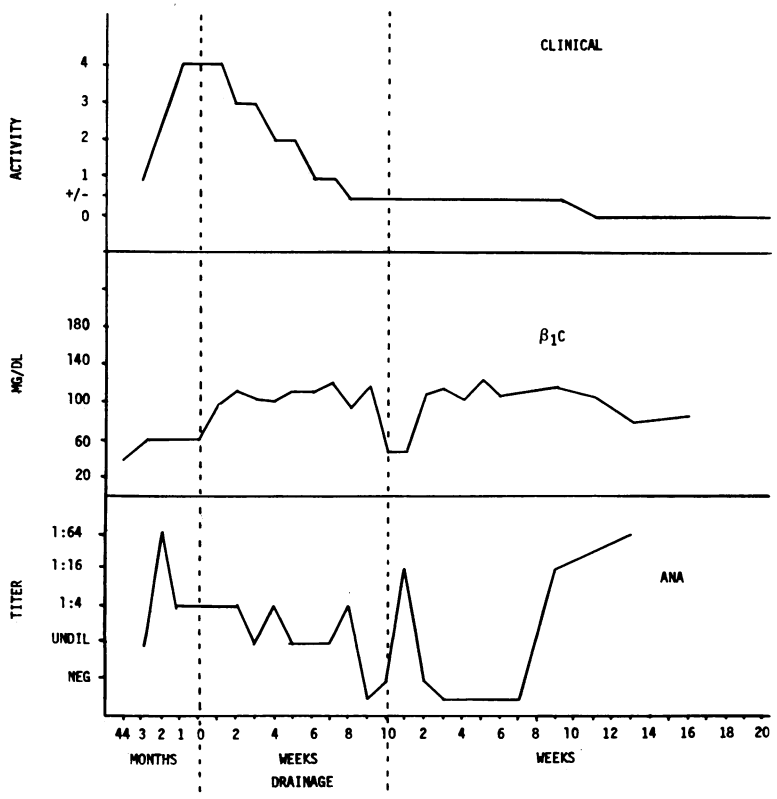

Figure 6.—Clinical activity of cutaneous vasculitis (arbitrary activity scale) and serum concentrations of Beta-1-C (β_1C) component of complement and antinuclear antibody (ANA) before, during and after thoracic-duct drainage.

were no lesions available for biopsy procedures. Similar distribution and intensity in staining for IgG were present in all specimens. Deposits of IgG and β_1C in uninvolved skin changed from continuous to focal with some decrease in intensity. Staining for IgM did not occur in the lesion or in uninvolved skin. Decreased deposition of IgG and β_1C closely paralleled the clinical course, whereas the antinuclear antibody (ANA) titer did not (Figure 6).

As expected in a patient with active lupus dermatitis, serum β_1C was notably depressed initially, as it had been for four years, in a range from 40 to 60 mg per dl. It rose immediately after initiation of drainage, anticipating the subsequent clinical improvement. By contrast, antinuclear antibody titer did not fall during the first eight weeks of drainage and became negative only at the end of the drainage, when the patient was significantly immunosuppressed.

IgG concentration decreased during thoracic-duct drainage and increased at the cessation of drainage. The results for IgA were surprising; it was undetectable before and early in drainage, but became detectable late in drainage and has since remained so. IgM concentrations fell during drainage, in contrast with the findings in patients with rheumatoid arthritis.

Thoracic-duct drainage was also used as a non-cytotoxic immunosuppressive therapy in a 22-year-old man with systemic lupus erythematosus manifested primarily by proteinuria (10 to 20 grams per day), recurrent superficial thrombophlebitis and cutaneous ulceration. From January 1972, he had been on a regimen of prednisone, 100 mg per day, and azathioprine, 150 mg per day, and had not responded to these drugs. Renal biopsy studies showed proliferative glomerulonephritis. Immunohistologic studies showed positive immunofluorescence in a fine, diffuse pattern for IgG (3+), IgM (2+) and β_1C (4+). On January 2, 1974, the thoracic duct was surgically cannulated. During drainage there was reduction in 24-hour urinary protein loss from 10 to 20 grams per day to 3 to 4 grams per day (Figure 7). During this period, the level of serum protein did not change. In addition, the dosage of oral corticosteroids was tapered from 70 mg of prednisone a day to 55 mg every other day, alternating with 2.5 mg every other day. Findings of postdrainage renal biopsy studies were identical to those of predrainage biopsy studies. In September 1975, aza-

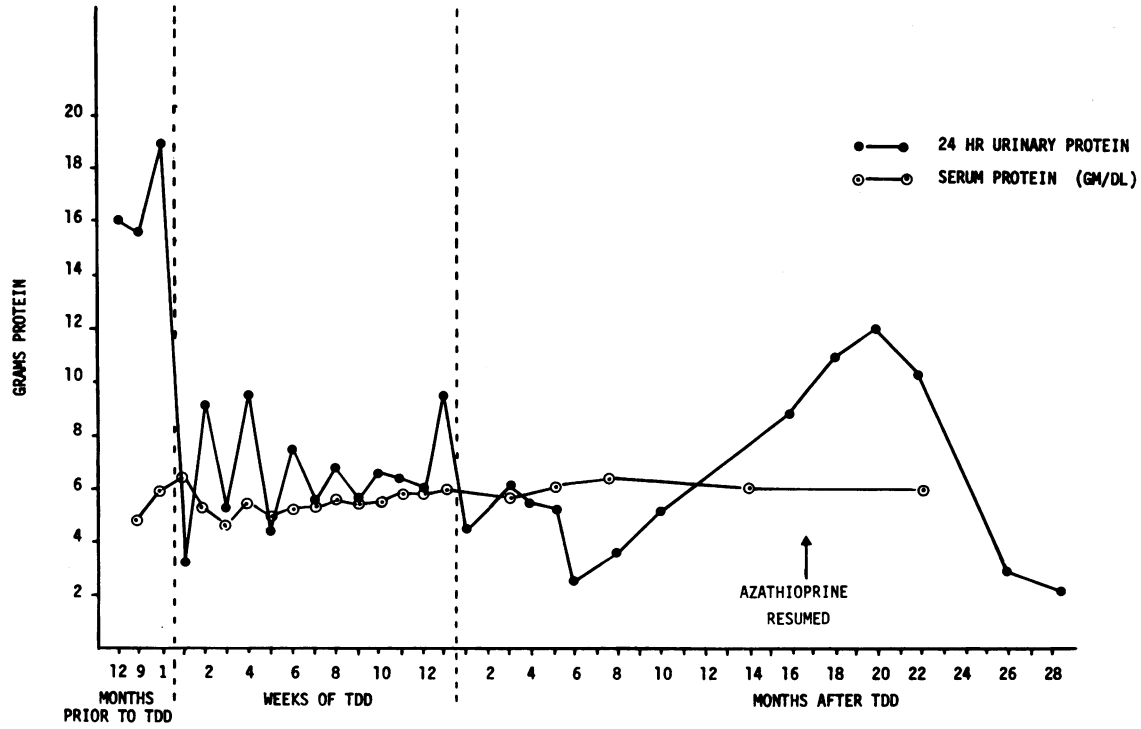

Figure 7.—Urine and serum protein concentrations before, during and after thoracic-duct drainage (TDD) in a patient with systemic lupus erythematosus.

thioprine was started again for decreasing renal function. At present he is receiving azathioprine, 300 mg per day, and prednisone, 50 mg every other day. During thoracic-duct drainage there seems to have been no histologic or immunohistologic change in renal tissue, although there was significant reduction in proteinuria.

The events during thoracic-duct drainage in systemic lupus erythematosus seem to follow this sequence: Initially there is clinical improvement associated with increases in total complement and β_1C; at this time immunosuppression is not present. With further diversion of thoracic-duct lymphocytes, there is a decrease in the deposition of "immune complexes" associated with further clinical improvement and little, if any, immunosuppression. With prolonged drainage, pronounced immunosuppression occurs, as shown by the feasibility of tapering dosages of corticosteroids and the prolonged skin-graft survivals; this is associated with decreasing titers of antinuclear antibody, which eventually become negative.

In summary, there was a prompt anti-inflammatory effect from diversion of thoracic-duct lymphocytes, as manifested by pronounced clinical improvement and rise of complement levels with no evidence of immunosuppression. With prolonged drainage, there was further clinical improvement and decreased "immune complex" deposition in skin; antinuclear antibody became negative; pronounced immunosuppression was shown by prolonged skin-graft viability, and it was possible to reduce corticosteroid doses.

We conclude that some lymphocytes circulating in the thoracic duct are important participants in processes leading to cutaneous vasculitis in systemic lupus erythematosus.

Rheumatoid Arthritis

SEYMOUR LEVINE, MD:* Lymphocytes are thought to play a role in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis. They are a prominent component of the infiltrates of inflammatory cells characteristically found in rheumatoid synovium and rheumatoid nodules. In order to study the effects of lymphocyte depletion on the clinical course of rheumatoid arthritis, we removed the lymphocytes circulating in the thoracic-duct lymph through a surgical fistula in nine patients with rheumatoid arthritis.[40]

Studies were done in 12 women with severe dis-

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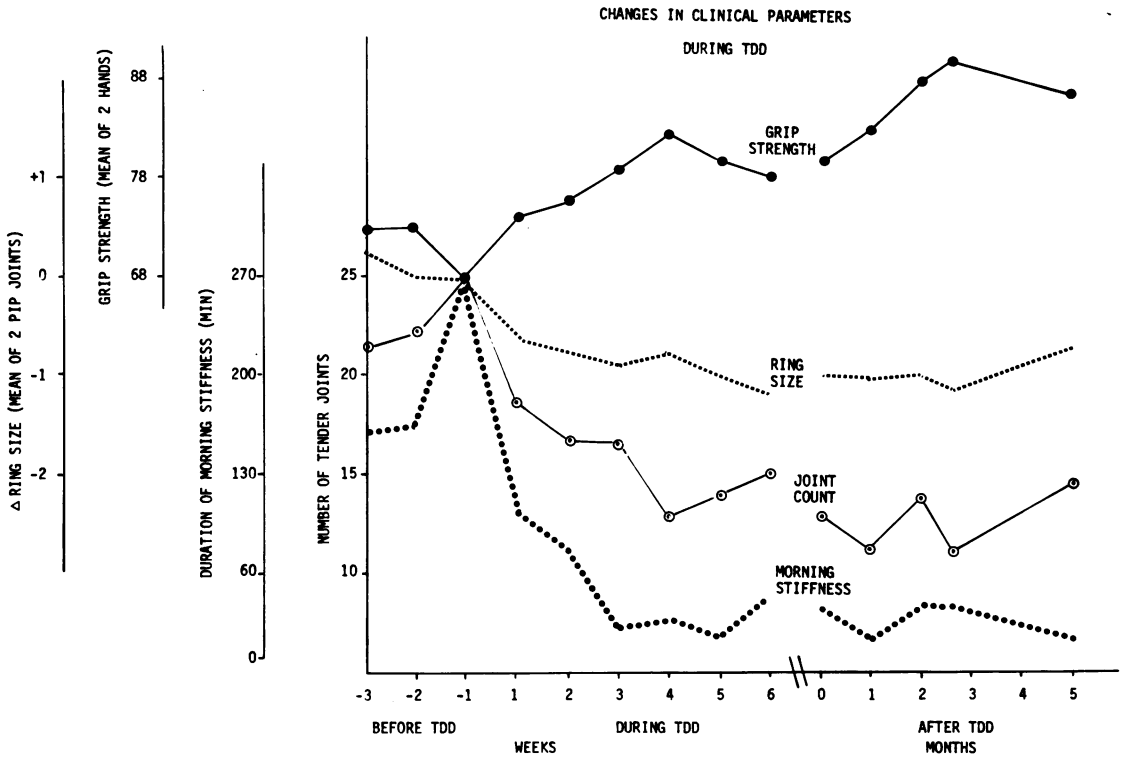

Figure 8.—Mean changes in clinical variables before, during and after thoracic-duct drainage (TDD) in nine patients with rheumatoid arthritis.

abling rheumatoid arthritis. In all there were high titers of rheumatoid factor, and in most patients the tests for antinuclear antibodies were positive; all but one had nodules and most had complicated disease with extraarticular manifestations. The thoracic ducts were successfully cannulated in nine patients and drained for 19 to 105 days (mean 53.4±10.8 days), during which 17×10^{10} to 111×10^{10} (mean $46\times10^{10}\pm12\times10^{10}$) lymphocytes were removed. In four patients fistulae were not established successfully, although the patients were subjected to the same general anesthesia and surgical procedures as those in whom cannulation was successful. These four patients were considered to be a comparison group. One patient was included in both groups; an initial attempt at cannulation was unsuccessful, but a second attempt six months later resulted in 105 days of lymph drainage.

Patients were admitted to hospital in the Clinical Research Center at UCLA for at least two weeks before cannulation to permit the patient and the disease to adjust to the hospital environment and to expedite baseline observations. Clinical variables followed each week included counting the number of joints with tenderness or pain on motion; determining maximum grip strength with a sphygmomanometer cuff; measuring the sizes of two preselected proximal interphalangeal joints with jeweler's rings, and recording the duration of morning stiffness.

In most patients administration of all anti-inflammatory drugs, including aspirin, was discontinued two or three days before operation; use of codeine or propoxyphene was permitted as needed for pain relief during drainage. Two patients continued to receive small doses of prednisone, and one patient continued to receive 150 mg of indomethacin per day during drainage.

Subjective improvement in the symptoms of arthritis began within seven days after institution of effective drainage, and measurable objective improvement was noted shortly thereafter. Statistically, significant improvement was noted when various measurements of disease activity were compared with baseline observations (Figure 8). Before drainage, patients' conditions became worse when anti-inflammatory drugs were withdrawn. After establishment of a thoracic-duct fistula, average count of painful joints dropped from 25 to 15 after six weeks of drainage. Similar improvement was seen for morning stiffness, with a drop from more than four hours to 40 minutes;

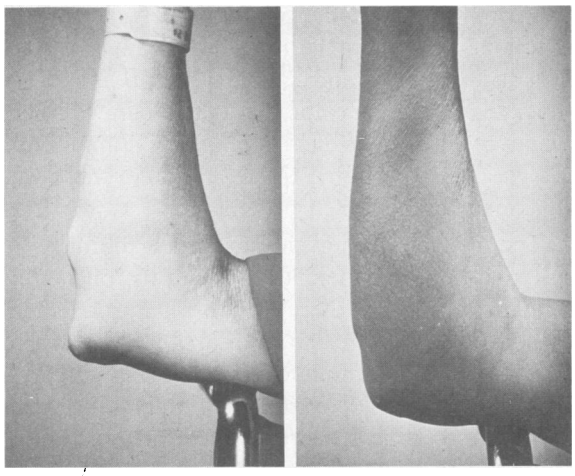

Figure 9.—Decrease in size of rheumatoid nodules in the olecranon bursa and on the extensor surface of the forearm during about five weeks of thoracic-duct drainage. (From Pearson, Paulus, Machleder;[41] reproduced by permission of the New York Academy of Sciences.)

for grip strength, with an increase from 67 to 78 mm of mercury; and for ring size, with a mean decrease of 1.2 units. With reinstitution of therapy with anti-inflammatory drugs, it was noted that improvement in patients' conditions lasted as long as five months after thoracic-duct drainage.

Improvement was only temporary, however, and evidence of active arthritis recurred within 2 to 12 weeks after drainage was discontinued. Nevertheless, in some patients disease activity was less severe and more easily controlled than before the study. The degree of improvement increased during prolonged drainage, and the duration of improvement after drainage seemed to increase with a longer period of drainage. Rheumatoid nodules decreased in size or disappeared during drainage (Figure 9).[41] The erythrocyte sedimentation rate became normal in three patients, but showed little change in the others. Latex titers showed no significant changes.

No improvement occurred after the four unsuccessful attempts at thoracic-duct cannulation, indicating that admission to hospital, anesthesia and surgical therapy were not responsible for the clinical improvement. In the one patient included in both groups, no clinical improvement occurred after unsuccessful cannulation. When successful cannulation was achieved six months later, however, clinical improvement occurred similar to that seen with the other patients in whom drainage was done. This patient's clinical status deteriorated when indomethacin was withdrawn before the initial attempt at cannulation of the thoracic

duct. After the unsuccessful surgical operation and resumption of indomethacin, there was a return to baseline in number of active joints and morning stiffness; however, the patient's condition gradually deteriorated. Six months later cannulation was done successfully and 10^{12} lymphocytes were removed during 105 days of thoracic-duct drainage. Subsequently, prolonged improvement occurred that lasted for nine months after drainage was stopped.

In summary, significant clinical improvement and pronounced suppression of disease activity occurred in nine patients with severe rheumatoid arthritis during prolonged continuous removal of thoracic-duct lymphocytes through a surgical fistula. The reasons for suppression of disease activity are not clear from these studies, nor is the specific role of these lymphocytes in the pathophysiology of the disease delineated by these clinical observations. It is unlikely that lymphocyte depletion affected the underlying cause of the disease, because active arthritis always recurred at some time subsequent to drainage. These findings do, however, suggest that some of the lymphocytes in the thoracic-duct lymph are essential for the continued activity of the inflammation associated with rheumatoid arthritis.

Lymphocyte reinfusion studies

In order to test this hypothesis, the reinfusion, both intravenously and intraarticularly, of autologous lymphocytes labeled with chromium 51 was studied in four patients.[40] With this method we hoped to assess clinical changes that might be associated with the reinfusion of these lymphocytes as well as to study lymphocyte recirculation.

Intravenous infusion of 1×10^9 autologous ^{51}Cr-labeled live lymphocytes into one patient on day 82 of drainage resulted in a generalized mild exacerbation of arthritis lasting seven to ten days. Such a flare-up did not occur after injection of a similar number of heat-killed cells (Figure 10). A moderate exacerbation of arthritis also occurred with infusion of 1×10^9 live cells into a second patient on day 58 of drainage. No such flare-up occurred when a similar number of live cells were infused into a third patient on day 2 of drainage; however, no improvement in arthritis had yet occurred so soon after thoracic-duct cannulation.

In order to eliminate the technical effects of washing and labeling of the lymphocytes with chromium 51, uncentrifuged whole lymph was reinfused into a fourth patient on three occasions,

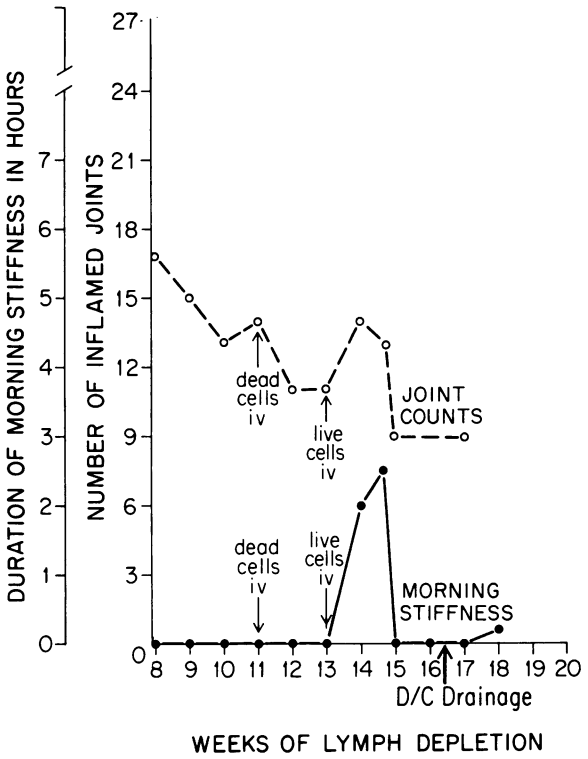

Figure 10.—Exacerbation of morning stiffness and number of inflamed joints after intravenous infusion of 1×10^9 living autologous thoracic-duct lymphocytes during thoracic-duct drainage. Infusion of the same number of dead autologous lymphocytes had no adverse effect.

days 24, 31 and 45 of drainage. Although this patient was informed that lymphocytes would be reinfused at some time, she was totally unaware of the exact days when this was carried out. Exacerbation of arthritis occurred three or four days after cell infusion and was documented by a threefold increase in joint count on two of the three occasions.

Intraarticular injection of 1×10^9 live lymphocytes into the left knee after 15 days of drainage resulted in an acute effusion and persistent inflammation of that joint. An increase in time needed to walk 50 feet from 19 to 29 seconds was associated with the acute inflammation of the knee. On the other hand, no such flare-up occurred when 1×10^9 *dead* cells were injected into the right knee of the same patient on day 21 of drainage.

Distribution of reinfused lymphocytes labeled with chromium 51

After intravenous infusion of labeled living lymphocytes, a tenfold increase of surface gamma counts was noted over the liver and spleen,

whereas much smaller increases occurred over the inflamed knees. Surface counts decreased exponentially during the next four weeks, but the rate of decrease was much slower over the knees than over the liver and spleen.

About 1 percent to 2 percent of the injected radioactivity appeared in the lymph during each day of the first week after injection of live cells. Of this label 40 percent to 50 percent was cell-associated, showing recirculation of labeled lymphocytes from blood to lymph. After injection of heat-killed labeled cells intravenously, no cell-associated radioactivity was found in the lymph. When labeled live lymphocytes were injected into the knee, cell-associated label also appeared in the thoracic-duct lymph. Most of the radioactivity remained in the injected knee, with a small amount detected over the liver or spleen, suggesting that lymphocytes leave the synovial space by way of lymphatics and the thoracic duct, rather than by directly entering the blood.

Finally, autoradiography showed only a few labeled lymphocytes in lymph, blood, synovial fluid and synovium. The slower rate of disappearance of radioactivity from the arthritic knees than from the liver and spleen may show that there is prolonged retention of some lymphocytes in the inflamed joint; control data for normal subjects are not available for comparison.

In summary, these studies showed that lymphocytes labeled with chromium 51 circulate from blood to thoracic duct. The label was widely distributed and could be followed using a gamma counter. Most of the radioactivity went to the liver and to the spleen; relatively little radioactivity went to inflamed joints after intravenous injection. Some labeled cells, however, could be identified in synovium and synovial fluid by autoradiography, indicating perhaps a "homing" mechanism of selected lymphocytes for joints. Some of the lymphocytes injected into a knee joint entered the thoracic duct. Reinfusing live cells intravenously or intraarticularly exacerbated the disease; this did not occur with dead cells. This exacerbation of inflammation further supports a role for some of these lymphocytes in the pathogenesis of rheumatoid arthritis and directly shows a detrimental role of lymphocytes in rheumatoid arthritis.

Summary

DR. PAULUS: To return to our original hypothesis, that we might be able to distinguish effects of thoracic-duct lymphocyte diversion from those of recirculating lymphocyte depletion, we can sum up (Table 5) as follows:

TABLE 5.—*Summary of Effects of Thoracic-Duct Drainage*

Diversion of Thoracic-Duct Lymphocytes
↓ Synovitis in rheumatoid arthritis
↓ Rheumatoid nodules in rheumatoid arthritis
↓ Cutaneous vasculitis in systemic lupus erythematosus
↓ Serum IgG
Depletion of Lymphocytes During Prolonged Thoracic-Duct Drainage
Pronounced ↓ recirculating lymphocyte pool
Pronounced ↓ cell-mediated immunity
Prolonged allograft survival
Selective ↓ humoral immune responses
No effect on induced inflammation
↓ T cells; ↑ B cells

Diversion of thoracic-duct lymphocytes produced a prompt decrease in synovitis and nodule size in rheumatoid arthritis, a diminution of cutaneous vasculitis and proteinuria in systemic lupus erythematosus and a pronounced drop in IgG serum concentrations.

Prolonged thoracic-duct drainage with *depletion* of recirculating lymphocytes was associated with a pronounced decrease in cell-mediated immunity, prolonged skin-graft survival and selective suppression of humoral antibody responses. Thoracic-duct T cells were depleted slightly more than B cells, and there was no effect on responses to induced inflammation. Additional improvement of clinical manifestations also occurred during depletion.

Substantial clinical improvement, although temporary, occurred in these patients with severe rheumatoid arthritis or systemic lupus erythematosus, who had failed to respond to previous therapy. This improvement occurred despite the withdrawal of nonsteroidal anti-inflammatory drugs in the patients with rheumatoid arthritis and the reduction of prednisone doses in the patients with systemic lupus erythematosus. It is consistent with a hypothesis that some of the cells removed from the lymph were essential participants in the inflammatory manifestations of rheumatoid arthritis and systemic lupus erythematosus in these patients. This hypothesis is supported by the exacerbation of inflammation that sometimes occurred three to seven days after we returned the patient's live thoracic-duct lymphocytes intravenously or intraarticularly. The studies using labeling with chromium 51 showed that a few of the reinfused live lymphocytes ended up in in-

flamed synovium and synovial fluid, but they did not show whether these cells play an important part in the inflammatory process. Exacerbation of arthritis occurred fairly soon after drainages of less than four weeks, but remissions were somewhat longer after prolonged depletion; this may show that prolonged depletion temporarily exhausts the supply of a critical subpopulation of lymphocytes, whereas briefer drainage merely diverts these critical cells away from a required pattern of circulation.

Improvement of rheumatoid arthritis during thoracic-duct drainage has been reported by Dumont, Mayer, and Mulholland[42] in one patient; Edgren and associates[43] in two patients; and Wegelius and co-workers[44] in five of six patients. Olhagen and Franksson[45] have reported thoracic-duct drainage in systemic lupus erythematosus. Clinical improvement has also been reported during thoracic-duct drainage of patients with myasthenia gravis,[46,47] multiple sclerosis,[48,49] sympathetic ophthalmia[48] and progressive glomerulonephritis.[50]

Several complications have occurred both in patients in whom drainage was done successfully and in those in whom it was not successful. Septicemia due to contamination of a batch of preservative-free heparin required termination of drainage in our first two patients. A staphylococcal wound infection prevented satisfactory drainage of one of the control patients; she had many vasculitic skin ulcers that were infected with the same organism. Candida vini was found during routine daily cultures of lymph in the patient with lupus cutaneous vasculitis. Although this had not caused symptoms, drainage was stopped and the patient was treated with amphotericin intravenously.

Pleuropericarditis occurred in one patient after four weeks of drainage when synovitis was substantially improved. A similar pleuropericarditis occurred in one of the control patients who was not drained. A platelet count that gradually dropped to 60,000 per cu mm prompted us to stop drainage in one patient. Severe neutropenia that developed slowly in one patient after 70 days of drainage ultimately responded to splenectomy. Splenectomy for the Felty syndrome had also been done in one patient seven years before drainage. In three of the control patients herpes zoster later developed, and in three other patients hemorrhagic cystitis occurred in association with cyclophosphamide therapy.

Weighing the risks against the benefits, we think that most of our patients have benefited from thoracic-duct drainage and the careful medical management that they received. However, we do not recommend the routine application of this procedure for the treatment of severe rheumatoid arthritis or systemic lupus erythematosus; thoracic-duct drainage is a relatively complex and mechanically demanding procedure that requires scrupulous attention to detail by the nursing and technical team during a long, expensive hospital stay. If cytotoxic drugs are not given shortly after the end of thoracic-duct drainage, the effects on both immune responses and disease manifestations are only temporary. Nevertheless, thoracic-duct drainage provides a useful quantitative tool for studying the effects of lymphocyte diversion and depletion on various manifestations of presumably autoimmune diseases. It may give us insight into the pathogenesis of some of these diseases. It provides clinical justification for *in vitro* and animal investigations of lymphocyte function and suggests that drugs that specifically affect lymphocyte function may be useful in these diseases.

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